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BIO-EFFICACY OF SYSTEMIC FUNGICIDES AGAINST *PHYTOPHTHORA* INFECTION IN BLACK PEPPER (*PIPER NIGRUM* L.)

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ABSTRACT

The bio-efficacy of systemic fungicides viz., Metalaxyl, Fosetyl Al, Ethazole, Oxadixyl and Propamocarb was evaluated for their effects on the different phases of *Phytophthora capsici*. Of these, first three fungicides along with Bordeaux mixture have been evaluated for their efficacy in checking the disease under field conditions. Metalaxyl-Ziram and Fosetyl Al fungicides were superior to Bordeaux mixture in reducing the disease. The results obtained are discussed.

INTRODUCTION

Black pepper (*Piper nigrum* L.) often referred to as 'King of Spices' is an important spice crop cultivated over 1.58 lakh hectares in the South Indian states of Kerala, Karnataka and Tamil Nadu. During 1987-88, the country earned about Rs. 2400 millions by way of exports of this spice.

Of all the maladies afflicting black pepper, infection caused by *Phytophthora capsici* is the most destructive. The estimated losses due to this disease range from 3.7 to 9.4% of the crop corresponding to losses of 119 to 905 metric tonnes of the produce, in Calicut and Kannoor districts of Kerala alone (Balakrishnan *et al*., 1986 Anandaraj *et al*., 1989).

The present chemical control measures include foliar spray and swabbing the collar portions of the vines with 1 and 10 per cent Bordeaux mixture respectively besides drenching the soil either with 1% Bordeaux mixture or 0.2% copper oxychloride. In view of the lack of host resistance among the popular cultivars of black pepper, fungicides are the most important means of disease control at present. However, the protectants that are used fail to offer satisfactory control of the disease quite often mainly because of (i) the soil-borne nature of the pathogen and its inaccessibility to fungicidal action because of survival in deeper layers of soil, infecting roots and collar portions of the vine, (ii) loss of fungicides applied to soil and plant due to leaching under heavy rainfall, and (iii) severe incidence of the disease because of fast spreading pathogen, congenial microclimate and availability of abundant suscept tissue.

Systemic fungicides presently available against *Phytophthora* diseases in other crops are known to be highly effective (Schwinn, 1983; Cohen and Coffey, 1986). However information available on the efficacies of these fungicides in controlling black pepper *Phytophthora* is highly inadequate. The objective of the present investigations is to evaluate the effect of some of the available anti-oomycete fungicides against *P. capsici* both *in* vitro and *in vivo*.

MATERIALS AND METHODS

The studies were conducted in three phases :

I *In vitro* experiments
   i. The effects of systemic fungicides on developmental stages of *P. capsici*.
   ii. Influence of pH on fungicidal activity
   iii. Compatibility of metalaxyl with insecticides (endosulfan and quinalphos) and nematicides (phorate and furadan).

II Green house experiments
   i. The efficacy of systemic fungicides in suppressing soil-borne inoculum.
   ii. Effect of post-infection treatment on lesion

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expansion.

III. Field experiments

i. The efficacy of ethazole, fosetyl-Al, metalaxyl and Bordeaux mixture in controlling infections of *P. capsici*.

ii. Persistence of metalaxyl in soil and plants and its mobility in soil

iii. Estimation of metalaxyl residues in black pepper.

RESULTS AND DISCUSSION

I. *In Vitro* Studies

i. Effect on mycelial growth

The inhibitory effects of five fungicides namely ethazole, fosetyl-Al, metalaxyl, oxadixyl and propamocarb on different developmental stages of the foot-rot pathogen, *P. capsici* were studied. Ethazole and metalaxyl were the most effective fungicides on mycelial growth of the fungus. Their ED_{50} values were 0.73 and 1.02 µg/ml on cornmeal agar and 0.91 and 0.44 µg/ml on carrot agar respectively. Though propamocarb had a low ED_{50} value (1.16 µg/ml) on cornmeal agar, it could not completely inhibit the growth of the fungus even at 1000 µg/ml. However, on carrot agar, its ED_{50} and minimal inhibition concentrations were quite high (284 and > 1500 µg/ml). The ED_{50} values of fosetyle-Al and oxadixyl were 61 and 25.5 µg/ml on cornmeal agar compared to 392 and 261.5 µg/ml on carrot agar respectively. There were considerable variations with regard to the fungitoxic activities on cornmeal agar and carrot agar, probably due to the differences in the nutrient status of these media.

Among the hyphal abnormalities induced by the fungicides the most prominent were thickening, excessive branching and coiling of hyphal tips. However, with propamocarb, these abnormalities were not noticed. The colonies of *P. capsici* were thick and puffy on medium amended with propamocarb. Vacuolization of hypha was also noticed in many cases.

ii. Effect on asexual reproduction

In the presence of 100 µg/ml each of metalaxyl, fosetyl-Al and ethazole, the fungus failed to produce any sporangia. However, the subsequent stages of the fungus namely indirect germination of sporangia and germination of encysted zoospores were less sensitive generally. Even at 1000 µg/ml concentration, none of the fungicides could inhibit zoospore release. The inhibitory effect of fosetyl-Al on zoospore release was found superior to those of other fungicides at lower test concentrations. Similar results have been reported by Farih *et al.* (1981).

The fungicides could induce many qualitative changes (like indirect germination and rupturing of sporangia). Direct germination and rupturing of sporangia were very common in treatments with fosetyl-Al, oxadixyl and ethazole. At 10 µg/ml of fosetyl-Al, about 90 per cent of the sporangia germinated directly. However, sporangia were found to be normal in metalaxyl and propamocarb treatments.

iii. Effect of pH on fungitoxicity

The activities of two of the fungicides namely fosetyl-Al and propamocarb were pH dependent. Fosetyl-Al was less effective in the alkaline medium (pH 9-10). This phenomenon could be attributed to the inactivation of the active principle of fosetyl-Al namely phosphorous acid (Cohen and Coffey, 1986). Propamocarb the other hand was found to lose its fungitoxicity in the acidic medium. These are in agreement with the results obtained by Papavizas *et al.* (1978).

iv Compatibility of metalaxyl with insecticides and nematicides

The pesticides commonly used in black pepper, cultivation, viz., endosulfan and quinalphos showed considerable fungitoxicity to mycelial growth and sporangiogenesis in P. capsici. The ED_{50} values for mycelial growth of P. capsici were 56 and 52 µg/ml for endosulfan and 58 and 276 µg/ml for qinalphos in cornmeal agar and carrot agar respectively. Sporangial production was inhibited by 89.5 and 92.4 per cent respectively at 150 µg/ml of endosulfan and quinalphos.

The fungitoxic and insecticidal properties of these chemicals individually and their mixtures in different proportions were estimated through bio-assays using

P. capsici and the 'pollu' beetle *Longitarsus nigripennis* as test organisms. The results showed no loss of properties of either metalaxyl or the insecticides when mixed. Metalaxyl showed synergistic interaction with endosulfan at 1:8 ratio, with regard to fungitoxicity, whereas the effects were additive with quihalphos (Table I). The bio-assay using 'pollu' beetle - *L. nigripennis* showed that there was no loss in the insecticidal properties of endosulfan and quinalphos as a result of mixing with metalaxyl. At all the concentrations of the mixtures tested, mortality of the insects was more than their mortality at corresponding concentrations of insecticides alone. GLC studies also further confirmed the compatibility of these insecticides with metalaxyl. Metalaxyl was also found compatible with furadan and phorate which are used in the control of nematode infections in black pepper.

II. Green house experiments

i) Effect on soil-borne inoculum

Using a simple seedling bioassay procedure, the efficacies of the systemic fungicides in suppressing soil-borne inoculum of *P. capsici* were evaluated. Two phenylamide fungicides, metalaxyl and oxadixyl were found highly effective when applied as soil drenches. Metalaxyl at 100 µg/ml (constituting 20 µg/g of dry soil) offered total protection to black pepper seedlings planted in fungicide-treated soil heavily contaminated with *P. capsici*. The mortality of seedlings in soil treated with 1000µg/ml of oxadixyl was only 5%. At 1000 µg/ml each of fosetyl-Al and propamocarb, the seedling mortalities were 7.5 and 40 per cent respectively compared to 100 per cent mortality in untreated control.

ii. Effect of post-infection treatment with fungicides

None of the five fungicides could prevent the expansion of lesions when applied on established lesions of *P. capsici* on black pepper leaves two days after inoculation. However, the highest inhibition of 87.9% was obtained with metalaxyl at 200 µg/ml (Table II) Fosetyl-Al, ethazole, oxadixyl and propamocarb each at 3000 µg/ml could inhibit the lesion expansion by 52.9, 55.4, 47.3 and 47.9 per cent respectively. Cohen *et al.* (1979) found that metalaxyl prevented the lesion production only if applied one or two days after inoculation.

III. Field experiments

i) Field performance of fungicides in disease control

Experiments were conducted at CPCRI Research

Table I. Interaction of metalaxyl with endosulfan and quinalphos as shown by the fungitoxicity of the mixtures to *Phytophthora capsici* in cornmeal agar

Ratios of fungicide and insecticide in the mixture	ED$_{50}$ values (µg/ml) Theoretical	ED$_{50}$ values (µg/ml) Observed	Interaction ratios	Type of interaction
Metalaxyl + Endosulfan				
1:1	2.00	2.01	0.99	additive
1:4	4.75	3.97	1.19	additive
1:8	8.02	4.50	1.78	synergistic
1:10	9.49	6.47	1.46	additive
Metalaxyl + Quinalphos				
1:1	2.00	0.85	2.35	synergistic
1:4	4.88	2.23	2.19	synergistic
1?8	8.06	5.05	1.59	additive
1:10	9.63	5.26	1.82	synergistic

ED$_{50}$ values of metalaxyl = 1.02 µg/ml; endosulfan = 56.1 µg/ml; quinalphos = 58.4 µg/ml.

Table II. Inhibition (%) of lesions of *Phytophthora capsici* on black pepper leaves following post-infection application of fungicides

Fungicide and concentration (µg/ml)	Increase in mean lesion size (m.m.) after 2 days)	Inhibition (%)
Ethazole		
100	11.60	26.25
1000	9.76	37.95
2000	9.40	40.24
3000	7.00	55.49
Fosetyl-Al		
100	14.15	10.04
1000	11.35	27.84
2000	9.81	37.63
3000	7.40	52.95
Metalaxyl		
1	11.70	25.61
10	11.72	25.49
100	6.93	55.94
200	1.90	87.92
Oxadixyl		
100	13.09	16.78
1000	11.68	25.72
2000	8.86	43.64
3000	8.27	47.39
Propamocarb		
100	9.44	39.94
1000	9.05	42.46
2000	7.34	53.33
3000	8.18	47.99
Control	15.73	00.00

Centre, Kannara, Thrissoor district, and a private garden Thiruvambady in Kozhikode district to evaluate the performance of metalaxyl-ziram, fosetyl-Al and ethazole in comparison with Bordeaux mixture in controlling *Phytophthora* infections in black pepper both in pure crop and arecanut-black pepper mixed cropping systems during 1984-86. The intensity of foliar infection and mortality of the vines were the lowest in plants treated with metalaxyl-ziram and fosetyl-Al irrespective of the cropping systems (Table III). In mixed cropping system, the mean percentages of vines dead were 1.66 and 3.51 in plants treated with metalaxyl-ziram and fosetyl-Al compared to 11.09, 7.4 and 11.50 per cent in vines treated with ethazole, Bordeaux mixture and untreated control respectively. In another experiment, soil application of two doses of metalaxyl granules (5% a.i) namely 20 and 10 g per vine were compared with Bordeaux mixture treatment. Application of metalaxyl granules at 20 g/vine resulted in the lowest incidence (3.3%) of the disease compared to 17.8% and 27.5% incidence in Bordeaux mixture treated and untreated control plots respectively.

ii. Persistence of metalaxyl

Using bio-assay, the activity of metalaxyl was detected in the soil and black pepper leaves at 10-day intervals after treatment. The activity of the fungicide could be detected for a longer period in leaves compared to that in soil. When applied at 40 g/vine, the activity of the fungicide could be detected even after 50 days in the leaves whereas in soil, it was negligible after 20 days (Table-IV). At the lower dose of 20 g/vine, the activity of the fungicide was noticed even after 30 days in leaves. However, in soil, its activity after 20 days was not significant. This might be due to the depletion of the fungicide in the soil because of continuous uptake by the plant and also because of microbial degradation (Bailey and Coffey, 1986; Zheng *et al.*, 1989).

iii. Residue analysis

Samples of black pepper collected from metalaxyl treated vines, four and six months after fungicide application were analysed using gas chromatographic procedures. The fungicide was not detectable in any of the samples. This may be due to long time interval between fungicide application and harvest, low dose of fungicide applied, and the hot and dry weather prevalent during harvest season (Carbas et al., 1987).

Taking into consideration, the low frequency

Table III. Mortality of black pepper vines under different fungicidal treatments against *Phytophthora capsici* at Kannara and Thiruvambady during 1984-1986

Fungicide	Mortality of vines (%)							
	Kannara				Thiruvambady			
	1984	1985	1986	Mean	1984	1985	1986	Mean
Bordeaux mixture	5.0	5.60	11.60	7.40	0.00	6.60	6.85	4.48
Ethazole	5.0	5.60	22.67	11.09	0.00	6.66	5.30	3.98
Fosetyl-A1	5.0	0.00	5.55	3.51	0.00	8.33	1.66	3.33
Metalaxyl ziram	0.0	0.00	5.00	1.66	1.60	0.00	0.00	0.53
Control	15.0	4.15	15.60	11.50	3.33	15.70	2.08	7.03

SEm = 2.74, CD at 5% = 7.82 SEm = 2.06 CD at 5% = 5.83

Table IV. Persistence of metalaxyl activity in black pepper plants and in soil as shown by growth inhibition of *Phytophthora capsici* on leaves and on cornmeal agar (CMA) containing soil extracts

Duration after treatment (days)	Lesion inhibition on leaves (%)		Growth inhibition on CMA + soil extract (%)	
	20 g/vine	40g/vine	20g/vine	40g/vine
10	83.6	87.3	45.03	45.33
20	65.2	79.8	25.5	27.36
30	23.4	43.9	2.2	6.4
40	8.16	28.26	0.13	3.2
50	5.06	19.16	0.4	0.4

SEM = 5.00 CD at 5% = 14.76 SEm = 4.64 CD at 5% = 13.69

of application, high efficacy in reducing the infections and absence of metalaxyl residues in detectable levels in black pepper, the use of metalaxyl-ziram has been suggested as a major component in the integrated management of *Phytophthora* infections in black pepper.

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